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Required frequency

Annually an MDL study must be performed for each combination of the following:

- Matrix (*if the solid and aqueous matrix methods are identical, extrapolation from the water MDL is allowable*)
- Preparatory Method
- Analysis Method
- Analyte (where an MDL study is appropriate)

Matrix – Prep Method – Analysis Method – Analyte (or group)

MDL studies can be completed by preparing and analyzing at least two MDL study replicates each quarter. Annually, these quarterly replicate results could then be used for the LOD determination.

NR 149.48 (2) (d) includes a provision for verifying "*the continued applicability of a previously determined limit of detection by an established and defensible protocol*". However, the program has reviewed a number of protocols and none of them has been deemed to be "defensible". We continue to explore different protocols for verifying that an established LOD remains valid, but at this time the logical conclusion is that the only defensible approach is to simply repeat the determination.

The MDL study passes if both the low and high spike criteria are satisfied:

- LOD < spike concentration, **and**
- LOD is not less than 10% of spike concentration

If both MDL studies were conducted properly and the resultant MDL still does not pass or the lab feels the passing MDL is not realistic – protocol is provided on how to establish a realistic, determinative LOD (MDL).

Reporting

Many parameters used in covered programs under NR 149 require that sample results be reported to the LOD. When reporting results to the LOD, the LOQ must also be reported.

The LOD and LOQ should be below the regulatory limits established by covered programs and project plans, where it is achievable. In some cases a more sensitive method may be required in order to meet these limits.

Why does WI require LOD/LOQ reporting? This requirement comes from the covered programs. The covered programs use data to make environmental impact assessments. The determination of whether or not an action level has been exceeded or whether additional monitoring is required is based on the sample results relative to the LOD and LOQ.

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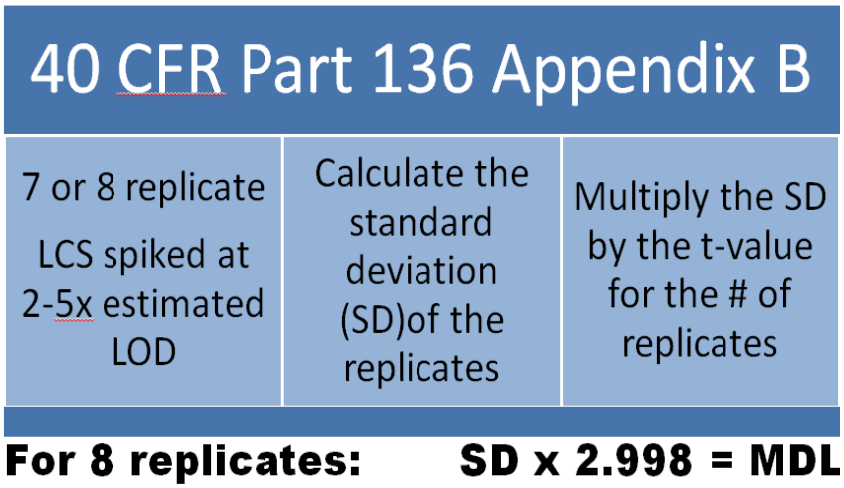
MDL (LOD) determination

- Begin with establishing a valid calibration.
- Before you attempt the MDL study it is in your best interest to take the time to review the 40 CFR Appendix B MDL procedures for how to determine a good estimated LOD.
- Most MDL study failures can be attributed to spiking at the wrong concentration and analyzing the replicates back to back in a single analytical run.
- The better you do estimating the spike concentration the better your chances are that you pass the MDL study the first time.
- The MDL procedure requires that a spike concentration be used in the MDL study that will be close to the estimated MDL (the procedure refers to 1x – 5x your current LOD). For example, using 2 - 3 times your current MDL concentration would be a good idea.
- If there is not a current MDL, or you suspect your MDL is no longer valid, there are two approaches you can use to determine a good estimated LOD:
    - 40 CFR Appendix B MDL procedures provide instruction on how to estimate a MDL.
    - Use your knowledge on instrument limitations, or the knowledge provided by other sources, such as the instrument vendor or an authoritative reference method.
- Perform the minimum 7 (but we encourage 8) replicate MDL study in 40 CFR Part, Appendix B.
    - Replicates should be analyzed over multiple days (run ~2 MDL spike samples each time). This is critical to incorporate day-to-day variability into the determination. Running all replicates simultaneously will frequently result in a failed MDL determination. It advisable to analyze the replicates distributed among routine samples, not directly after a blank.
- As previously discussed, the MDL study passes if it meets both of the following criteria:
    - LOD < spike concentration, **and**
    - LOD is not less than 10% of spike concentration



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Example 1: MDL (LOD) determination meets criteria

EXAMPLE 1: **A valid MDL determination**

LOD/LOQ Calculation and Validation Worksheet 6-24-09

Test:	Arsenic by 200.9	Date:	
		Analyst	

Spike Level:	2.000
Units	ug/L

INSTRUCTIONS

MDL Study Sample Number	Result	% Recovery
Replicate 1	2.14	107%
Replicate 2	2.11	106%
Replicate 3	1.9	95%
Replicate 4	1.7	85%
Replicate 5	1.62	81%
Replicate 6	2.07	104%
Replicate 7	1.92	96%
Replicate 8		0%
Replicate 9		0%
Replicate 10		0%
Average:	1.923	96%
Standard Deviation:	0.202	
Student's t-value to use:	3.143	
Calculated LOD:	0.6	ug/L
Calculated LOQ (10/3LOD):	2.1	

# Replicates	Student's t-value
7	3.143
8	2.998
9	2.896
10	2.821

☑ LOD < spike *[0.6 < 2 ug/L]*

☑ LOD > 10% spike *[0.6 > 0.2 ug/L]*

In Example 1, a valid LOD is obtained. Unless you have documentation to substantiate otherwise, this would become your determinative LOD.

What do i do if the first mdl study attempt fails?

- If the initial MDL study does not pass then adjust the spike concentration based on the study results and re-perform the study one more time.
- Before you make your 2nd attempt at the MDL study it is in your best interest to take the time to review the MDL procedure for how to determine a good estimated LOD.
- It is a very good idea to determine at what concentration a standard can be seen that can be distinguished from a blank.
- This is done by analyzing lower and lower concentration standards (serial dilutions)
- Once you find the concentration where a result is detectable (3x – 5x greater than the signal/noise or 3x the standard deviation of a set of blanks) you have determined an estimated LOD.
- Then take this estimated LOD value and redo the MDL study using standards at 3x the LOD, or if the instrument is highly precise it may be preferable to lower the MDL

study to standards that are at, or just above, this detected level. Again you will achieve the best chance of passing your MDL study by making sure variability is accounted for by running different study samples over a number of days.

EXAMPLE 2: Initial determination fails criteria; 2nd attempt passes

LOD/LOQ Calculation and Validation Worksheet Rev 1, 3/2015

Test:	Arsenic by 200.9	Date:	
		Analyst	

Spike Level:	5.000	2.000
Units	ug/L	ug/L

		Initial attempt	2nd attempt
MDL Study Sample Number	Result	% Recovery	Result
Replicate 1	5.110	102%	2.140
Replicate 2	5.060	101%	2.110
Replicate 3	4.900	98%	1.900
Replicate 4	4.900	98%	1.700
Replicate 5	5.180	104%	1.620
Replicate 6	4.910	98%	2.070
Replicate 7	4.780	96%	1.920
Replicate 8	5.020	100%	
Replicate 9			
Replicate 10			
# replicates	8		7
Average:	4.983 (99.65%)		1.923 (96.1429%)
Standard Deviation:	0.132		0.202
Student's t-value to use:	2.998		3.143
Calculated LOD:	0.40		0.64
Calculated LOQ (10/3LOD):	1.32		2.12

ok LOD < spike? [0.4 < 5 ug/L] ok LOD < spike? [0.64 < 2 ug/L]
X LOD >10% spike? FAILS ok LOD >10% spike? [0.6 > 0.2 ug/L]

In Example 2, on the second attempt a valid LOD is obtained.

This is often the case when the initial spiking concentration was just too high. Remember that our target is to spike closer to the LOD, where quantitation is less accurate, and the standard deviation of replicates increases.

Unless you have documentation to substantiate otherwise, this would become your determinative LOD.

But what if, after two attempts at performing the LOD determination, you still don't meet criteria or the nominal LOD is unrealistically low?

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*An alternate approach to determining a realistic, determinative **LOD***

If the lab has addressed the issues in the first MDL study…

- by re-determining a good estimate of the LOD and
- by spreading out replicate analyses to account for additional variability and
- the 2nd MDL study still fails criteria or results in an unrealistically low LOD

…then it is time to use an alternate approach to establish a realistic, determined LOD.

This is usually only applicable for high precision instruments *(and typically for those in which there are no sample preparation steps)*, such as for ion chromatography and flow injection analysis.

Step 1: Demonstrate that the nominal LOD is unrealistic

The first step in using a value other than that obtained using the approved EPA protocol, as your determinative LOD, is to demonstrate that the nominal LOD determined is not realistic.

One way to do this is to analyze a processed LCS prepared at a concentration equal to the nominal LOD and demonstrate that either there is no signal or the quantitative result is below the nominal LOD.

Another way to do this is to demonstrate that the nominal LOD response is not significantly greater (three times or higher) than the response found in routine method blanks.

Step 2: Rule out blank contamination or poor low end characterization of the calibration

Answer the questions,

- How do you know that you do not have low level contamination?
- Are your blanks "negative?
- How do you know the calibration levels and algorithm are not contributing to blank concerns?

Step 3: Analyze several LCS samples spiked at levels above the nominal LOD

Once certain that blank levels are not adversely affected by contamination or calibration issues, one can analyze several LCS samples at increasing concentrations, starting at the LOD concentration. Each LCS is evaluated for the instrument's ability to detect it at its concentration based on instrument response. Be certain that the result LCS response can be distinguished from blank responses (i.e. not a false positive).

Remember that quantitative recovery is difficult to reliably achieve between the LOD and the LOQ. Your "determinative LOD" is the lowest concentration at which you can reliably detect a signal that is not a false positive. If you get good recovery, that's even better.

Make sure that the LCS concentrations used are not significantly higher than the nominal LOD. concentration.

Be sure that you can answer the question, "How are you certain that the determinative LOD is not lower?"

Step 4: Establish the LOQ

The LOQ can be calculated as 10/3 x the LOD.

Example 3: **A valid alternative LOD determination**

LOD/LOQ Calculation and Validation Worksheet 6-24-09

Test:	Mercury by 245.1		Date:	
			Analyst	

Spike Level:	0.500		0.100	
Units	ug/L		ug/L	
Blank result	0.022		0.027	
		Initial attempt	2nd attempt	
MDL Study Sample Number	Result	% Recovery	Result	
Replicate 1	0.488	98%	0.110	
Replicate 2	0.503	101%	0.108	
Replicate 3	0.492	98%	0.098	
Replicate 4	0.476	95%	0.112	
Replicate 5	0.514	103%	0.115	
Replicate 6	0.509	102%	0.100	
Replicate 7	0.491	98%	0.112	
Replicate 8	0.487	97%	0.105	
Replicate 9		0%		
Replicate 10		0%		
# replicates	8		8	
Average:	0.495	99%	0.108	108%
Standard Deviation:	0.013		0.006	
Student's t-value to use:	2.998		2.998	
Calculated LOD:	0.04	ug/L	0.02	
Calculated LOQ (10/3LOD):	0.13		0.06	

ok	LOD < spike	[0 < 0.5 ug/L]	ok LOD < spike [0 < 0.1 ug/L]
X	LOD > 10% spike	FAILS	ok LOD > 10% spike [0 > 0 ug/L]

Alternative approach

Analyzed LCS	0.02	
Obtained	0.009	ND
Recovery	45.0%	
Analyzed LCS	0.035	
Obtained	0.029	
Recovery	82.9%	
Analyzed LCS	0.05	
Obtained	0.051	
Recovery	102.0%	
Typical blank		
response	0.0012	
concentration	0.025	

SDWA MCL	2
SDWA LOD	0.2
NR 140 PAL	0.2
NR 140 ES	2

- In Example, 3, the lab performed an LOD that failed criteria.
- The study was repeated at a lower level, which met criteria.
- Unfortunately, the resultant LOD falls below typical levels observed in blanks.
- An LCS prepared at the nominal LOD was undetectable demonstrating that the nominal LOD was unrealistic.
- A second LCS, at 0.035 ppb, was within the normal variance for blanks. Therefore 0.035 ppb would not meet the definition of an LOD which is designed to avoid false positives.
  Finally, an LCS at 0.05 ppb was associated with a response well above that observed in blanks.
- In addition, 0.05 ppb is still well below relevant regulatory limits.
- Therefore, this protocol established a valid determinative LOD at 0.05 ppb.

Example 4: **Insufficient alternative LOD impacted by calibration.**

LOD/LOQ Calculation and Validation Worksheet 6-24-09

Test:	Arsenic by SM 3113B		Date:		
			Analyst		
Spike Level:	8.000		4.000		Alternative approach
Units	ug/L		ug/L		
Blank result	-0.596		-0.183		
				Analyzed LCS	1.0
		Initial attempt	2nd attempt	Obtained	0.753
MDL Study Sample Number	Result	% Recovery	Result	Recovery	75.3%
Replicate 1	7.774	97%	3.46		
Replicate 2	7.874	98%	3.72		
Replicate 3	7.963	100%	3.6	Analyzed LCS	3.2
Replicate 4	7.567	95%	3.7	Obtained	3.304
Replicate 5	7.684	96%	3.81	Recovery	103.3%
Replicate 6	7.672	96%	3.92		
Replicate 7	7.703	96%	3.85		
Replicate 8		0%			
Replicate 9		0%			
Replicate 10		0%			
# replicates	7		7		
Average:	7.748	97%	3.714	#DIV/0!	
Standard Deviation:	0.134		0.162		
Student's t-value to use:	3.143		3.143		
Calculated LOD:	0.4	ug/L	0.5	ug/L	
Calculated LOQ (10/3LOD):	1.4		1.7		
	ok LOD < spike	[0.4 < 8 ug/L]	ok LOD < spike	[0.5 < 4 ug/L]	
	X LOD > 10% spike	FAILS	ok LOD > 10% spike	[0.5 > 0.4 ug/L]	

- In example 4, the initial nominal LOD (0.4 ppb) was rejected due to failed criteria.
- The second attempt met criteria and yielded a nominal LOD (0.5 ppb) very close to that obtained initially. That would seem to corroborate a determinative LOD of about 0.5 ppb.
- However, the lab rejected the second nominal LOD as unrealistic and opted to employ an "alternative approach" to establishing its determinative LOD.
- The lab subsequently prepared an LOD verification standard at 1.0 ppb and an LOQ verification at 3.2 ppb. Since good recoveries were observed for both standards, the lab established its LOD at 1 and LOQ at 3.2.
- The decision to start at 1.0 ug/L as the "determinative LOD" may have been related to the fact that the required SDWA LOD for arsenic is 1 ug/L or less.
- The rationale for ignoring the acceptable 2nd LOD determination was based on its historical experience that method blanks averaged a concentration of -0.5 ppb with a standard deviation of 0.6.
- That would be an appropriate rationale as long as the lab could demonstrate that their calibration is not adversely impacting blank values.

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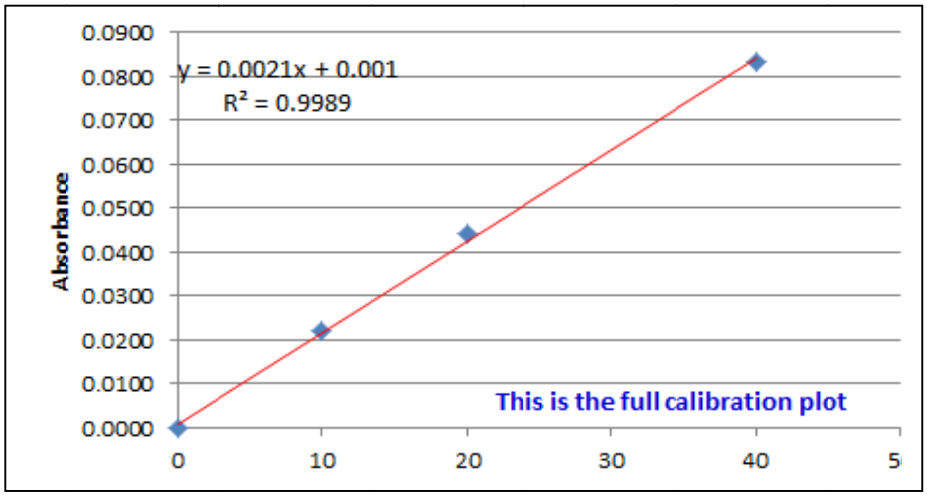
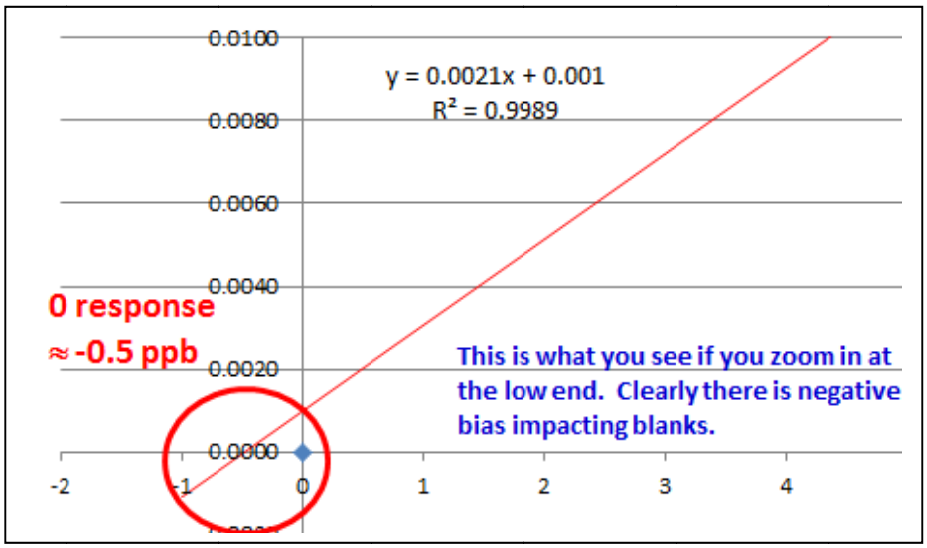
The question unanswered is: How do we know that the determinative LOD doesn't fall between 0.5 and 1.0? No analysis was performed in this region.

And why are blanks routinely negative with significant variance? What's wrong with this picture?

Subsequently, the source of the negative blank bias was investigated. On closer examination, the lab was calibrating with standard levels of 0, 10, 20, and 40 ppb. Clearly, this is an inappropriate calibration, even for an LOD of 1.0. Remember, the first calibration standard needs to be near the LOQ. Furthermore, how often would one expect to find arsenic in drinking water above even 10 ppb? Sure, it happens in some areas, but calibrations must be designed to cover the normal range of anticipated sample concentrations and must include adequate definition of the range near the LOQ.

With an LOD of 0.5 ppb, a more appropriate calibration would be 0, 2, 5, 10, and 20 ppb.





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LOQ determination

The LOQ must be mathematically related to the LOD (just indicating that the LOQ is greater than the LOD is not a mathematical relationship).

The traditionally accepted statistical definition of the LOQ is 10/3 the LOD.

The lowest calibration standard

NR 149.44(6)(e) requires that, "*Laboratories reporting results at levels at or near the limit of detection of an analysis shall include in initial calibrations a standard at a concentration near the limit of quantitation of the analysis*."

Most analyses performed for "covered programs" of the agency will require results to be reported down to the LOD. Therefore, calibrations need to include a standard "near" the LOQ. The further the standard concentration is from the actual LOD, the more significant the potential impact on achieving a realistic, determinative LOD.

Additional information and examples for MDL studies are included in a document called Analytical Detection Limit Guidance on our website:
http://dnr.wi.gov/regulations/labcert/documents/guidance/-LODguide.pdf

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Definitions

Method blank = reagent water that is processed simultaneously with and under the same conditions as the associated samples – including all preparatory, cleanup, and analysis steps.

LOD (Limit of detection) = the lowest concentration or amount of analyte that can be identified, measured, and reported with confidence that the concentration is not a false positive value. For department purposes, the LOD approximates the EPA's MDL (method detection limit) and is determined according to the protocol established in 40 CFR Part 136, Appendix B. A quantitative recovery is not expected at the LOD. The study must be well done because the LOD is used to set the LOQ and for some parameters determining a true LOD is very important to the programs using that data.

> **Nominal LOD** = the LOD calculated following the accepted EPA protocol (statistically derived). It remains the Nominal LOD until it has been properly vetted or replaced with either a vetted 2nd determination, or one determined through some alternative protocol, acceptable to the Department.
>
> **Determinative LOD** = the adopted LOD used for reporting. The adopted LOD may be the LOD obtained from the initial MDL study, the 2nd determination, or the one determined from an approved alternate protocol.
>
> **Method Detection Limit** (MDL) = the minimum concentration of an analyte that can be measured and reported with 99% confidence that the stated concentration is greater than zero as determined from analyses of a set of samples containing the analyte in reagent water. The method detection limit is generated according to the protocol specified in 40 CFR 136, Appendix B. It is listed here because most EPA methods refer to the MDL.

LOQ (Limit of quantitation) = the lowest concentration or amount of an analyte for which quantitative results can be obtained. A quantitative recovery is expected at the LOQ. NR 149 requires that there be a mathematical relationship between the LOD and the LOQ. Traditional statistics define the LOQ as 10/3 the LOD.

Lowest concentration standard in the calibration curve = NR 149 requires that the lowest standard in the initial calibration be near the LOQ. "Near" could be defined as 2-5 times the LOQ for multi-analyte methods. Note however that the combination of 5 times the LOQ and the LOQ being 10/3 of the LOD means that the low calibration standard could be as much as 17 times the LOD. This situation should be avoided as much as possible as it could lead to bias at the low end of the calibration curve (see Example 4).

> **NOTE:** *Calibration is arguably the most critical part of determining an LOD. The calibration must be properly established without over-extending the upper limit of the calibration, while also properly characterizing the low end of the calibration. The predominance of "negative" blanks is a primary indicator of a poor calibration.*
>
> ***Reminder: the calibration levels and calibration algorithm selected for the LOD determination MUST be the same as that used for analysis of samples and QC.***